...TENT COOPERATION TREA...

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 27 April 2000 (27.04.00)	
International application No.: PCT/AU99/00896	Applicant's or agent's file reference: 91958
International filing date: 18 October 1999 (18.10.99)	Priority date: 16 October 1998 (16.10.98)
Applicant: KEEGAN, Mitchell et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

20 January 2000 (20.01.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 91958	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU99/00896**	International Filing Date *(day/month/year)* 18 October 1999	Priority Date *(day/month/year)* 16 October 1998
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12N 15/63, 15/70, 15/85, 15/79		
Applicant COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **8** sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[X]	Certain observations on the international application

Date of submission of the demand 20 January 2000	Date of completion of the report 25 January 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **GILLIAN ALLEN** Telephone No. (02) 6283 2266

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU99/00896

I. Basis of the rep rt

1. With regard to the **elements** of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-14** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages **16,**as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **15, 17** received on **5/1/01** with the letter of **5/1/01**
- [X] the drawings, pages **3/13-13/13** as originally filed,
  pages , filed with the demand,
  pages **1/13 and 2/13,** received on **11/4/00** with the letter of **11/4/00**
- [X] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages **1/4 - 4/4** received on **11/4/00** with the letter of **11/4/00**

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

- [X] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1-27**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-27**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-27**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

1. Citations

D1. Cullen, Bryan R. Expression of a cloned human interleukin-2 DNA is enhanced by the substitution of a heterologous mRNA leader region. DNA. 1988. 7(9):645-650

2. Novelty

The applicant submits that Cullen et al use only part of the insulin secretory sequence in their Il-2 construct. This is accepted, and the claims are therefore novel over the prior art.

3. Inventive Step

Cullen et al do not use the entire insulin secretory sequence to improve expression of the Il-2 gene, however they do use the 6 amino acids from the N terminal of the insulin secretory sequence. The constructs of the present application include these 6 amino acids, as well as the remaining amino acids of the secretory sequence. However, Cullen et al appear to suggest that it is the structure and sequence of the 5' non coding region, which they term the "leader sequence", of the mRNA, rather than the encoded signal peptide leader sequence, that affects translation. In view of this teaching, although the constructs of the citation and the present invention share some features, the present claims are considered inventive.

Industrial Applicability.

All claims are considered industrially applicable

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The subject matter of the present claims was not considered to be supported by the description beyond constructs of the insulin signal sequence operably linked to somatotropin. There was no disclosure in the description as filed that the surprisingly increased secretion obtained with this construct would extend to constructs of the insulin signal peptide fused to other heterologous proteins. However, the applicant has provided supporting evidence that the insulin signal sequence fused to other heterologous proteins does lead to increased secretion of the heterologous peptide, so it is accepted that the effect of increased secretion is not limited to somatotropin.

Claims:

1. An expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

2. An expression cassette according to claim 1, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

3. An expression cassette according to claim 1, wherein the insulin secretory signal is a modified insulin secretory signal comprising modifications of the insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1, wherein said modifications do not deleteriously affect the biological activity of the insulin secretory signal.

4. An expression cassette according to any one of claims 1 to 3, wherein the heterologous sequence encodes a polypeptide selected from hormones, cytokines, receptor agonists, receptor antagonists, pheromones, and enzymes.

5. An expression cassette according to claim 4, wherein the polypeptide is a growth hormone.

6. An expression cassette according to claim 5, wherein the polypeptide is somatotropin.

7. An expression cassette according to any of claims 1 to 6, further including one or more regulatory elements to enable pulsatile expression of the heterologous sequence .

8. A vector including an expression cassette according to any one of claims 1 to 7.

9. A recombinant cell which includes an expression cassette according to any one of claims 1 to 7.

10. A recombinant cell according to claim 9, wherein the cell is a bacterial, yeast, insect or mammalian cell.

21. A method of administering somatotropin to a pig, wherein the method includes implanting in the pig a capsule including a semi-permeable membrane encapsulating recombinant cells, said recombinant cells including and expressing an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding somatotropin, wherein said membrane is permeable to the expresssed somatotropin.

22. A method according to claim 21, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

23. A method according to claim 21, wherein the insulin secretory signal is a modified insulin secretory signal comprising modifications of the insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1, wherein said modifications do not deleteriously affect the biological activity of the insulin secretory signal.

24. A method according to any one of claims 21 to 23, wherein the recombinant cells are mammalian cells.

25. A method according to claim 24, wherein the mammalian cells are rat myoblast (L6) cells.

26. A method according to any one of claims 21 to 25, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

27. A method according to any one of claims 21 to 26, wherein the pig is implanted with one or more capsules sufficient to achieve secretion of somatotropin of at least 30 ng/ml.

FIGURE 1: ISS-pST gene construct

```
   1  GCTAGCATGG CCCTGTGGAT GCGCCTCCTG CCCCTGCTGG CGCTGCTGGC
  51  CCTCTGGGGA CCTGACCCAG CCGCAGCCCT CGAGATGTTT CCAGCTATGC
 101  CACTTTCTTC TCTGTTCGCT AACGCTGTTC TTCGGGCCCA GCACCTGCAC
 151  CAACTGGCTG CCGACACCTA CAAGGAGTTT GAGCGCGCCT ACATCCCGGA
 201  GGGACAGAGG TACTCCATCC AGAACGCCCA GGCTGCCTTC TGCTTCTCGG
 251  AGACCATCCC GGCCCCCACG GGCAAGGACG AGGCCCAGCA GAGATCGGAC
 301  GTGGAGCTGC TGCGCTTCTC GCTGCTGCTC ATCCAGTCGT GGCTCGGGCC
 351  CGTGCAGTTC CTCAGCAGGG TCTTCACCAA CAGCCTGGTG TTTGGCACCT
 401  CAGACCGCGT CTACGAGAAG CTGAAGGACC TGGAGGAGGG CATCCAGGCC
 451  CTGATGCGGG AGCTGGAGGA TGGCAGCCCC CGGGCAGGAC AGATCCTCAA
 501  GCAAACCTAC GACAAATTTG ACACAAACTT GCGCAGTGAT GACGCGCTGC
 551  TTAAGAACTA CGGGCTGCTC TCCTGCTTCA AGAAGGACCT GCACAAGGCT
 601  GAGACATACC TGCGGGTCAT GAAGTGTCGC CGCTTCGTGG AGAGCAGCTG
 651  TGCCTTCTAG TCTAGA  (SEQ ID NO:4)
```

ATG...GCC- insulin secretory signal.

GCTAGC- *Nhe* I restriction site incorporated into construct in order to ligate into plasmid.

CTCGAG- *Xho* I restriction site incorporated into construct in order to ligate secretory signal and pST.

TCTAGA- *Xba* I restriction site incorporated into construct in order to ligate into plasmid.

FIGURE 2: ISS-pST peptide sequence.

```
  1  MALWMRLLPL LALLALWGPD PAAALEMFPA MPLSSLFANA VLRAQHLHQL
 51  AADTYKEFER AYIPEGQRYS IQNAQAAFCF SETIPAPTGK DEAQQRSDVE
101  LLRFSLLLIQ SWLGPVQFLS RVFTNSLVFG TSDRVYEKLK DLEEGIQALM
151  RELEDGSPRA GQILKQTYDK FDTNLRSDDA LLKNYGLLSC FKKDLHKAET
201  YLRVMKCRRF VESSCAF    (SEQ ID NO:3)
```

MAL....AAA- insulin secretory signal, cleaved upon secretion of pST.
LE- function of XhoI cleavage site; result in no predicted secondary structural changes to pST.

Sequence listing:

Applicants: Commonwealth Scientific and Industrial Research Organisation
University of Western Sydney (Nepean)
Pig Research and Development Corporation

Title of the Invention: Delivery system for porcine somatotropin

Prior Application Number: PP 6556
Prior Application Filing Date: 1998-10-16

Number of SEQ ID NOs: 4

Software: PatentIn Ver. 2.1

SEQ ID NO: 1
Length: 24
Type: PRT
Organism: Homo sapien

Sequence: 1

Met	Ala	Leu	Trp	Met	Arg	Leu	Leu	Pro	Leu	Leu	Ala	Leu	Leu	Ala	Leu
1				5				10						15	

Trp	Gly	Pro	Asp	Pro	Ala	Ala	Ala
		20					

SEQ ID NO: 2
Length: 72
Type: DNA
Organism: Homo sapien

Sequence: 2

atggccctgt ggatgcgcct cctgcccctg ctggcgctgc tggccctctg gggacctgac 60
ccagccgcag cc

SEQ ID NO: 3
Length: 666
Type: DNA
Organism: Artificial Sequence

Feature:
Other Information: Description of Artificial Sequence: ISS-pST gene construct

Sequence: 3

gctagcatgg ccctgtggat gcgcctcctg cccctgctgg cgctgctggc cctctgggga 60
cctgacccag ccgcagccct cgagatgttt ccagctatgc cactttcttc tctgttcgct 120
aacgctgttc ttcgggccca gcacctgcac caactggctg ccgacaccta caaggagttt 180
gagcgcgcct acatcccgga gggacagagg tactccatcc agaacgccca ggctgccttc 240
tgcttctcgg agaccatccc ggcccccacg ggcaaggacg aggcccagca gagatcggac 300
gtggagctgc tgcgcttctc gctgctgctc atccagtcgt ggctcgggcc cgtgcagttc 360
ctcagcaggg tcttcaccaa cagcctggtg tttggcacct cagaccgcgt ctacgagaag 420
ctgaaggacc tggaggaggg catccaggcc ctgatgcggg agctggagga tggcagcccc 480
cgggcaggac agatcctcaa gcaaacctac gacaaatttg acacaaactt gcgcagtgat 540
gacgcgctgc ttaagaacta cgggctgctc tcctgcttca agaaggacct gcacaaggct 600
gagacatacc tgcgggtcat gaagtgtcgc cgcttcgtgg agagcagctg tgccttctag 660
tctaga 666

SEQ ID NO: 4
Length: 217
Type: PRT
Organism: Artificial Sequence

Feature:
Other Information: Description of Artificial Sequence: ISS-pST peptide sequence

Sequence: 4

Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15

Trp Gly Pro Asp Pro Ala Ala Ala Leu Glu Met Phe Pro Ala Met Pro
20 25 30

Leu Ser Ser Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His
35 40 45

Gln Leu Ala Ala Asp Thr Tyr Lys Glu Phe Glu Arg Ala Tyr Ile Pro
50 55 60

Glu Gly Gln Arg Tyr Ser Ile Gln Asn Ala Gln Ala Ala Phe Cys Phe
65 70 75 80

Ser Glu Thr Ile Pro Ala Pro Thr Gly Lys Asp Glu Ala Gln Gln Arg
85 90 95

Ser Asp Val Glu Leu Leu Arg Phe Ser Leu Leu Leu Ile Gln Ser Trp
100 105 110

Leu Gly Pro Val Gln Phe Leu Ser Arg Val Phe Thr Asn Ser Leu Val
115 120 125

Phe Gly Thr Ser Asp Arg Val Tyr Glu Lys Leu Lys Asp Leu Glu Glu
130 135 140

Gly Ile Gln Ala Leu Met Arg Glu Leu Glu Asp Gly Ser Pro Arg Ala
145 150 155 160

Gly Gln Ile Leu Lys Gln Thr Tyr Asp Lys Phe Asp Thr Asn Leu Arg
165 170 175

Ser Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Ser Cys Phe Lys
180 185 190

Lys Asp Leu His Lys Ala Glu Thr Tyr Leu Arg Val Met Lys Cys Arg
195 200 205

Arg Phe Val Glu Ser Ser Cys Ala Phe
210 215

Claims:

1. An expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

2. An expression cassette according to claim 1, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

3. An expression cassette according to claim 1, wherein the insulin secretory signal is a modified insulin secretory signal having substantially the same overall biological activity as an insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1.

4. An expression cassette according to any one of claims 1 to 3, wherein the heterologous sequence encodes a polypeptide selected from hormones, cytokines, receptor agonists, receptor antagonists, pheromones, and enzymes.

5. An expression cassette according to claim 4, wherein the polypeptide is a growth hormone.

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7. An expression cassette according to any of claims 1 to 6, further including one or more regulatory elements to enable pulsatile expression of the heterologous sequence .

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10. A recombinant cell according to claim 9, wherein the cell is a bacterial, yeast, insect or mammalian cell.

21. A method of administering somatotropin to a pig, wherein the method includes implanting in the pig a capsule including a semi-permeable membrane encapsulating recombinant cells, said recombinant cells including and expressing an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding somatotropin, wherein said membrane is permeable to the expresssed somatotropin.

22. A method according to claim 21, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

23. A method according to claim 21, wherein the insulin secretory signal is a modified insulin secretory signal having substantially the same overall biological activity as an insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1.

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25. A method according to claim 24, wherein the mammalian cells are rat myoblast (L6) cells.

26. A method according to any one of claims 21 to 25, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

27. A method according to any one of claims 21 to 26, wherein the pig is implanted with one or more capsules sufficient to achieve secretion of somatotropin of at least 30 ng/ml.

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 101  CACTTTCTTC TCTGTTCGCT AACGCTGTTC TTCGGGCCCA GCACCTGCAC
 151  CAACTGGCTG CCGACACCTA CAAGGAGTTT GAGCGCGCCT ACATCCCGGA
 201  GGGACAGAGG TACTCCATCC AGAACGCCCA GGCTGCCTTC TGCTTCTCGG
 251  AGACCATCCC GGCCCCCACG GGCAAGGACG AGGCCCAGCA GAGATCGGAC
 301  GTGGAGCTGC TGCGCTTCTC GCTGCTGCTC ATCCAGTCGT GGCTCGGGCC
 351  CGTGCAGTTC CTCAGCAGGG TCTTCACCAA CAGCCTGGTG TTTGGCACCT
 401  CAGACCGCGT CTACGAGAAG CTGAAGGACC TGGAGGAGGG CATCCAGGCC
 451  CTGATGCGGG AGCTGGAGGA TGGCAGCCCC CGGGCAGGAC AGATCCTCAA
 501  GCAAACCTAC GACAAATTTG ACACAAACTT GCGCAGTGAT GACGCGCTGC
 551  TTAAGAACTA CGGGCTGCTC TCCTGCTTCA AGAAGGACCT GCACAAGGCT
 601  GAGACATACC TGCGGGTCAT GAAGTGTCGC CGCTTCGTGG AGAGCAGCTG
 651  TGCCTTCTAG TCTAGA  (SEQ ID NO:3)
```

ATG...GCC- insulin secretory signal.

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FIGURE 2: ISS-pST peptide sequence.

```
  1  MALWMRLLPL LALLALWGPD PAAALEMFPA MPLSSLFANA VLRAQHLHQL
 51  AADTYKEFER AYIPEGQRYS IQNAQAAFCF SETIPAPTGK DEAQQRSDVE
101  LLRFSLLLIQ SWLGPVQFLS RVFTNSLVFG TSDRVYEKLK DLEEGIQALM
151  RELEDGSPRA GQILKQTYDK FDTNLRSDDA LLKNYGLLSC FKKDLHKAET
201  YLRVMKCRRF VESSCAF    (SEQ ID NO:2)
```

MAL....AAA- insulin secretory signal, cleaved upon secretion of pST.
LE- function of XhoI cleavage site; result in no predicted secondary structural changes to pST.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 91958	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 99/00896	International filing date *(day/month/year)* 18 October 1999	(Earliest) Priority Date *(day/month/year)* 16 October 1998

Applicant

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION, UNIVERSITY OF WESTERN SYDNEY (NEPEAN) and PIG RESEARCH AND DEVELOPMENT CORPORATION

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention

☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00896

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/63, 15/70, 15/79, 15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
AS ABOVE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CA, MedLine
Insulin, signal peptide, gene expression, vector or cassette
Genbank, EMBL, PDB
SEQ ID 1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Cullen, Bryan R. Expression of a cloned human interleukin-2 DNA is enhanced by the substitution of a heterologous mRNA leader region. DNA. 1988. 7(9):645-650 V	1-20

☐ Further documents are listed in the continuation of Box C

☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 1999	-2 DEC 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer GILLIAN ALLEN Telephone No.: (02) 6283 2266

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/63, 15/70, 15/79, 15/85

A1

(11) International Publication Number: **WO 00/23601**

(43) International Publication Date: 27 April 2000 (27.04.00)

(21) International Application Number: PCT/AU99/00896

(22) International Filing Date: 18 October 1999 (18.10.99)

(30) Priority Data:
PP 6556 16 October 1998 (16.10.98) AU

(71) Applicants *(for all designated States except US)*: COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, ACT 2601 (AU). UNIVERSITY OF WESTERN SYDNEY (NEPEAN) [AU/AU]; Second Avenue, Kingswood, NSW 2747 (AU). PIG RESEARCH AND DEVELOPMENT CORPORATION [AU/AU]; 3rd floor, Computer Associates House, 10 National Circuit, Barton, ACT 2600 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KEEGAN, Mitchell [AU/AU]; 19/105–109 Albert Street, Werrington, NSW 2747 (AU). JONES, Mark, Richard [AU/AU]; 203 Tennyson Road, Tennyson, NSW 2754 (AU). MOORE, Geoffrey, Philip, M. [AU/AU]; 17 Carrington Street, Summer Hill, NSW 2130 (AU).

(74) Agent: F. B. RICE & CO.; 605 Darling Street, Balmain, NSW 2041 (AU).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN

(57) Abstract

An expression construct is disclosed which is useful for delivering an exogenous polypeptide (e.g. a growth hormone such as somatotropin) to a host. In one application of the invention, the expression construct is introduced into a non–host recombinant cell encapsulated in a semi–permeable membrane for implantation into the host. The semi–permeable membrane inhibits immune surveillance and cell rejection events so that non–host, highly expressing, recombinant cells can be used.

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DELIVERY SYSTEM FOR PORCINE SOMATOTROPIN

Field of the Invention:

The present invention relates to an expression construct for delivering an exogenous polypeptide to a host. The present invention also relates to recombinant cells which include this expression construct and to semi-permeable capsules which include the recombinant cells.

Background of the Invention:

In mammals, somatotropin (growth hormone) is normally secreted from the pituitary gland. However, exogenous administration of somatotropin to pigs has been shown to improve feed efficiency 15-20%, increase daily weight gain 10-15%, reduce carcass fat 10-20%, increase lean meat content 5-10% and reduce feed intake. Unfortunately, somatotropin (which is a small protein of 190 amino acids) is susceptible to gastric acids and protein digestion hence daily injections are required in order to be efficacious. Currently, welfare and ethical issues discourage the use of the pneumatic pST injection gun and the costs of daily administration restrict industry-wide adoption.

Recent advances in gene therapy have enabled the development of strategies which avoid the dependence on autologous target cells and immunosuppressive therapy by utilising transfected cells encapsulated in a semi-permeable alginate-poly-L-lysine-alginate (APA) membrane. The APA capsule environment is compatible with cell viability and growth so that transfected cells remain viable, secreting growth factors, for extended periods. The APA is permeable to small proteins and consequently gene expression can be controlled by external means. The APA barrier inhibits immune surveillance and cell rejection events so that non-host, highly expressing, cells can be employed in the capsule. The APA barrier may also prevent uncontrolled proliferation of the transfected cells in the recipient host. The APA capsule can be removed, potentially re-used, in order to negate the concerns regarding consumption of transgenic material. Further, if the capsule is damaged by severe tissue trauma a normal host-graft rejection would destroy the implanted cells.

Summary of the Invention:

The present inventors have now found that ligation of an insulin secretory signal to a heterologous gene sequence prior to introduction of the gene sequence into a host cell results in a surprising increase in the level of secretion of the heterologous gene product. This finding has led to the development of an improved gene delivery system involving encapsulation of recombinant cells for implantation into a host.

Accordingly, in a first aspect, the present invention provides an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

By "heterologous sequence" we mean a sequence other than a sequence encoding insulin.

By "operably linked" we mean that the insulin secretory signal sequence is contiguous and in reading frame with the heterologous coding sequence.

The preferred insulin secretory signal is an insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1. However, it will be appreciated by those skilled in the art that a number of modifications may be made to that secretory signal without deleteriously affecting the biological activity of the signal. For example, this may be achieved by various changes, such as sulfation, phosphorylation, nitration and halogenation; or by amino acid insertions, deletions and substitutions, either conservative or non-conservative (eg. D-amino acids, desamino acids) in the peptide sequence where such changes do not deleteriously affect the overall biological activity of the secretory signal. Thus, the inclusion in the expression cassette of an insulin secretory signal which has been modified in one or more of the abovementioned ways, is to be regarded as being encompassed by the present invention.

The heterologous sequence may encode any polypeptide, other than insulin, of interest. For example, the heterologous sequence may encode a hormone, cytokine, receptor agonist or antagonist, pheromone or enzyme. In a preferred embodiment, the heterologous sequence encodes a growth hormone. Preferably, the growth hormone is somatotropin.

In a second aspect, the present invention provides a vector including an expression cassette of the first aspect. The vector may be any suitable

vector for introducing the expression cassette into a cell. Suitable vectors include viral vectors and bacterial plasmids.

The expression cassette of the first aspect of the present invention, or the vector of the second aspect, may further include one or more elements which regulate gene expression. Examples of suitable regulatory elements include the Melatonin Response Element (MRE) (as described in Schrader *et al, 1996*, the entire contents of which are incorporated herein by reference), and/or rapamycin mediated transcription factors (as described in Magari *et al, 1997*, the entire contents of which are incorporated herein by reference). In a preferred embodiment, the regulatory element(s) enable pulsatile expression of the polypeptide of interest.

In a third aspect, the present invention provides a recombinant cell which includes an expression cassette according to the first aspect of the present invention.

The recombinant cell may be a bacterial, yeast, insect or mammalian cell. In a preferred embodiment, the recombinant cell is a mammalian cell. In a further preferred embodiment, the cell is a rat myoblast (L6) cell.

In a fourth aspect, the present invention provides a method of producing a polypeptide which includes culturing a recombinant cell of the third aspect under conditions enabling the expression and secretion of the polypeptide and optionally isolating the polypeptide.

The recombinant cell(s) of the present invention may be encapsulated in a semi-permeable matrix for delivery or implantation in a host.

Accordingly, in a fifth aspect, the present invention provides a capsule for implantation in a host, the capsule including a semi-permeable membrane which encapsulates one or more recombinant cells according to the third aspect of the present invention.

In a preferred embodiment, the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane. The preparation of an APA semi-permeable membrane is described in Basic *et al, 1996*, the entire contents of which are incorporated herein by reference.

In a sixth aspect, the present invention provides a method of administering a polypeptide to a host which includes administering to the host an expression cassette according to the first aspect of the present invention.

In a seventh aspect, the present invention provides a method of administering a polypeptide to a host which includes implanting in the host a capsule according to the fifth aspect of the present invention.

The host may be any animal or human. In a preferred embodiment, the host is a livestock animal. In a further preferred embodiment, the host is selected from the group consisting of grazing cattle, feed-lot cattle, dairy cows, pigs and poultry.

It will be appreciated by those skilled in the art that the present invention provides an improved system for the delivery of genetic material to a host. The ligation of the insulin secretory signal to a biologically active polypeptide leads to increased secretion of the polypeptide from recombinant cells. Following secretion, the secretory signal may be cleaved leaving the biologically active polypeptide. The recombinant cells, when encapsulated in a semi-permeable membrane, have the capacity to secrete significant amounts of the biologically active polypeptide and the semi-permeable membrane enables control of gene expression by external means. Implantation of the encapsulated recombinant cells provides an advantage in that the implantation requires minimal surgery. Further, the semi-permeable membrane reduces immune surveillance and cell rejection which means that non-host cells can be employed in the capsule.

In a preferred embodiment, the semi-permeable membrane is durable which provides an advantage in that it may limit cell growth thereby preventing uncontrolled proliferation in the recipient host. The capsules provide a further advantage in that they may be removed and re-used.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following non-limiting Examples and Figures.

Brief description of the accompanying figures:

Figure 1: Insulin secretory signal - pST gene construct.

Figure 2: Insulin secretory signal - pST peptide sequence.

Figure 3: Rate of weight gain (from day 0) for control and individual pST-L6IXS treated pigs.

Figure 4: Percentage weight gain for control and individual pST-L6IXS treated animals.

Figure 5: Plasma, pST levels for control and individual pST-L6IXS treated animals.

Figure 6: Plate 1- Appraisal of pST-L6IXS capsule administration site
Plate 2 - Placement of pST-L6IXS capsule in culture media for ex-vivo assessment.

Figure 7: Ex-vivo assessment of secretion of pST from capsules for a 24 hr period following removal from host animal.

Figure 8: Mean plasma pST (over 3 hours @ 30 min intervals) before (white bars) and 1 week post pST capsule administration (black bars) (*significant).

Figure 9: Daily plasma pST concentrations of two pigs, pig 206 and 228, with implanted capsules secreting 25 ng/ml and 500 ng/ml respectively.

Figure 10: Rate of Gain (ROG) in kg/day (black squares) and P2 back fat measurements in pigs produced in Example 4.

Figure 11: Rate of Gain (ROG) of male pigs following implantation with pST secreting or control immunoneutral gene therapy (IGT) capsules (± SEM).

Figure 12: Back fat (P2) of male pigs following implantation with pST secreting or control immunoneutral gene therapy (IGT) capsules (± SEM).

Figure 13: Loin (eye) muscle area of male pigs following implantation with pST secreting or control immunoneutral gene therapy (IGT) capsules (± SEM).

Detailed description of the invention:

Example 1: Cloning of the ISS-pST construct

The pST gene was obtained from Southern Cross Biotechnology Pty Ltd in an *E. coli* bacterium. The plasmid containing the pST gene, pMG939, was isolated from the bacterium using standard plasmid preparation techniques. The PCR primers were designed to amplify the pST gene, add an *Xho* I site to the 5' end and an *Xba* I site to the 3' end to enable ligation events.

The modified pST gene sequence was subsequently ligated to a secretory signal sequence (ISS) derived from the preproinsulin cDNA. *Nhe* I (GCTAGC) and *Xba* I (TCTAGA) restriction sites were constructed in front of the ISS start codon and after the 3' terminal codon of pST, respectively, to allow incorporation into the pCI-neo plasmid (Promega). The pST fusion construct was subsequently isolated and sequenced to verify the coding region (Figure 1).

Transfection of rat myoblast (L6) cells (pST gene incorporation into cells) was performed, with LipoTAXI (Stratagene), 2hrs after the L6 cells were trypsin treated. pST transfected L6 cell clones were maintained in culture, selected with G418, until $>10^7$ cells were generated. Aliquots (2ml) of the culture supernatant were stored at -20°C prior to assessment of pST concentrations in a pST radioimmunoassay (RIA) established by Dr P. Wynn at Sydney University (Camden). The RIA sensitivity was deemed to be >0.4ng/ml with CV's in the order of 12.4%. The polyclonal antisera was raised in guinea pigs with a pST peptide antigen. The RIA results (Table 1) indicate that the pST gene construct produced protein (Figure 2) which is recognised by polyclonal antisera raised against the native form of pST, purified from porcine pituitary glands. L6 Clones pCI/pst-1..5 were generated from the modified transfection technique as described below.

Modified transfection protocol

Characteristically, L6 cells adhere to culture plates and require detachment with trypsin to passage cells; transfection is routinely performed 24hrs later. This procedure resulted in L6 cell clones (n=10) secreting pST at 6-18 ng/ml. Applying LipoTAXI (Promega) and the ISS/pST plasmid to the L6 cells 2hrs after trypsin treatment increased the secretion rate of pST 10-20 fold (>180ng/ml, n=5 clones). This higher pST secretion rates reduce the number of cells (capsules) required to enhance growth.

TABLE 1: Concentrations (ng/ml) for each clone transfected with ISS-pST.

L6 clone	pST (ng/ml)
pCI/pst-1*	182
pCI/pst-2*	188
pCI/pst-3*	188
pCI/pst-4*	140
pCI/pst-5*	200
pCI/pst-6	17
pCI/pst-7	12
pCI/pst-8	8
pCI/pst-9	9
pCI/pst-10	7
pCI/pst-11	7
pCI/pst-12	10
pCI/pst-13	8
pCI/pst-14	6
pCI/pst-15	18

Example 2: Preparation of the porcine somatotropin-rat myoblast (L6) immunoneutral expression system (pST-L6IXS)

The encapsulation procedure described in Basic *et al, 1996*, was followed with the following modifications.

Encapsulation of cells at room temperature, utilises calcium chloride (or lactate) [100mM] to gel the alginate [1.5% w/v] droplets followed immediately by washing with saline (0.9% NaCl) then resuspending in poly-L-lysine [0.05%] for 5 min. Calcium chloride crosslinking for 10min at 37°C resulted in an alginate matrix that was more compatible with cell viablity.

After the poly-L-lysine coating and saline washes another alginate layer is added. Sodium citrate [55mM] treatment for 4min at room temperature softens the capsule to a consistency that increases the difficulty of further manipulation. Cell viablity is apparently reduced to <35% with 4 min exposure to sodium citrate. Placing the capsules in a cell strainer prior to sodium citrate treatment enabled 1min exposure, at 37°C, improving cell viability to >98%.

Procedural and equipment modifications to the encapsulation protocol improved the efficiency (time and resources) of encapsulation with routine increases in cell viability in the order of 64%.

Example 3: Pilot experiment (1) involving implantation of pST-L6IXS in pigs

Preliminary results obtained with the pST-L6IXS, administered to growing mice, indicate enhanced growth characteristics. In a pilot experiment with male pigs (n=9, mean live weight 61 kg) varying numbers of pST-L6IXS were administered in different sites (3 capsules, i.m. in the neck muscle, 3 capsules s.c. in the neck, 10 capsules s.c. at the base of the ear, 20 capsules i.m. in the neck or 29 capsules i.m. in the neck of individual animals on day 0). Blood samples (10ml) were collected via jugular venipuncture and P2 ultra-sound (us) measurements were recorded at -14, 0, 7, 14, 21, 28 and 36 days post administration. The sites of pST-L6IXS administration were monitored for tissue reaction events throughout the experiment. On day 36 animals were euthanased and carcass analysis (back fat depth, BF(mm); eyemuscle area, EMA(cm); forearm bone length, BONE(cm); heart weight, HEART(gm); spleen weight, SPLEEN(gm) and liver weight, LIVER(gm) were recorded (see Table 2) and pST-L6IXS recovered. Figure 3 represents the rate of gain (from day 0) for control (con, mean$\pm$SE, n=4) and individual values for pST-L6IXS treated pigs. Percentage weight gain, over the pST-L6IXS treatment is presented in Figure 4 with the mean$\pm$SE for control (con) pigs and individual pST-L6IXS treated animals. Plasma pST (ng/ml) was determined by radioimmunoassay (RIA) and presented in Figure 5, with mean$\pm$SE control (con) and individual concentrations for pST-L6IXS treated pigs. At slaughter the site of pST-L6IXS capsule administration was appraised (Figure 6, Plate 1, arrow) prior to removal and placement in culture media for ex-vivo assessment (Figure 6, Plate 2) of 24 hour secretion of pST (Figure 6). No apparent tissue damage or immune reactions were observed either i.m. or s.c. at day 36. However, the capsules placed in the ear (s.c.) appeared to be highly vascularised and were 100% recoverable. The capsules placed in the neck region were <10% recoverable.

The pST-L6IXS remained patent over 36 days *in vivo* and appeared to proliferate within the capsule (Plate 2) which can be removed in order to negate the concerns regarding consumption of transgenic material. Further, if the capsule is damaged (i.e. by severe tissue trauma) a normal host-graft rejection destroys the L6 cells preventing propagation of transfected material. Experiments in mice and pigs have demonstrated that pST-L6IXS are

efficacious in altering plasma pST, enhancing growth characteristics and potentially immune competence of animals.

TABLE 2

pST-L6IXS PILOT EXPERIMENT:
Pigs (male) supplied by Westmill piggery (Young, NSW)
Experiment at EMAI, maximum security piggery.
ACEC Ref No: 98/20

				LIVEWEIGHT (kg)													
				Date													
				###	##	###	##	###	###	9/07/98							
				Day						(slaughter)	CARCASS						
	Pen	Treatment	Animal	-14	0	7	14	21	28	36	P2us (mm)	BF (mm)	EMA (cm)	BONE (cm)	HEART (gm)	SPLEEN (gm)	LIVER (gm)
	A	con	291	24	67	NR	NR	89	95	100	11	9	54.5	24.5	388.6	159.8	1720.2
C	A	con	292	25	61	NR	NR	84	90	90	8	10	54.9	23.7	381.5	103.2	1703.6
C	B	con	294	22	74	NR	NR	94	103	104	12	15	46.5	24.4	391.5	173.2	1636.5
C	B	con	295	22	55	NR	NR	76	84	91	9	7	50.6	20.0	396.6	138.2	1561.8
T	B	3sc neck*	297	23	67	NR	NR	85	90	91	9	12	45.2	23.5	385.3	177.0	1817.7
		*infected capsule site								CvTp<0.05	nsd	nsd	CvTp<0.06	nsd	CvTp<0.05	nsd	nsd

Example 4: Pilot experiment (2) involving implantation of pST-L6IXS in pigs

A second pilot experiment was conducted in order to optimise pST-L6IXS delivery by capsules so as to achieve growth responses similar to the energy repartitioning observed with daily pST injections.

As shown in Example 1, pST secreting cells have been produced with a range of secretion rates (6-200 ng/ml). pST secretion rates in the order of 2-25 ng/ml appear to be the most stable following the imposition of stress (i.e. by bacterial contamination) on the pST secreting cells (data not shown). Accordingly, clones secreting about 5 ng/ml (clone pCI/pst-14) and about 10 ng/ml (pCI/pst-12) were selected for this pilot experiment. Male pigs (n=10, mean live weight 78.1 kg) were administered various numbers of capsules (produced according to the procedure described in Example 2) s.c. at the base of the ear (Table 3).

Pig	Capsule Number	Clone
204	1	a
216	1	b
230	3	a
202	3	b
226	5	a
206	5	b
208	10	a
224	10	b
222	100	a
228	100	b

a = clone pCI/pst- 14 (5 ng/ml)
b = clone pCI/pst-12 (10 ng/ml)

Body weights were recorded at the beginning and the end of the experiment. Animals were held in individual pens (2 m^2) and stabilised to a controlled environment facility (22^{0}C) for 1 week. The animals were offered *ad libitum* water and standard pelleted grower rations (3 kg/day @ 09:00 hrs),

and daily residues were recorded. Catheters were placed in ear veins (evc), and 24 hours later sampling commenced. Control pig (i.e. no pST capsules) blood plasma (10 ml) was collected every 30 min for 3 hours. pST capsules were administered to the ipsilateral ear immediately following serial sampling. Blood (10 ml) was collected via evc (daily @ 11:00 hrs) while catheters remained patent. Treatment (7 days post administration of pST capsules) blood plasma (10 ml) was collected every 30 min for 3 hours. Slaughter and carcass analysis was performed at about 100 kg live weight 21 days later. pST capsules were then recovered from ears and placed in *in vitro* culture (for pST assay). The capsule site was also assessed for immune responses (e.g. lymphocyte infiltration).

The results of measurements of mean (3 hr, 30 min interval) plasma pST concentration of pigs before and 7 days after receiving pST capsules (secreting between 5 and 1000 ng/ml) are shown in Figure 8. As can be seen from Figure 8, it is apparent that plasma pST is reduced in pigs following 1 week exposure to immunoneutral pST (5 - 100 ng/ml) secreting capsules.

The variability between and within individual plasma pST concentrations appeared to be more apparent during the control serial sampling period. This phenomenon is reflected in the Standard Errors about the mean observed concentrations. Further, the stable baseline and pST pulse intervals (normally 3 - 4 hrs) were not recognised by computer programs designed to identify hormone pulses. However, stable baselines and distinct pST pulses were observed in animals 1 week post pST casule administration (Figure 9).

The Rate of Gain (ROG) shown by the animals appeared to be responsive to pST capsule secretion in a dose dependent manner (Figure 10). A secretion rate of 30 ng/ml (i.e. 3 capsules secreting 10 ng/ml each) appears to be the minimum dose required to observe growth rate increases. The majority of evc's remained patent for 21 days at which time, the animals were euthanased with barbituate for carcass analysis. Analysis of carcass back fat (P2 without skin) measurements further indicate that 30 ng.ml is the minimum dose to observe energy repartitioning within 21 days of pST capsule administration (Figure 10).

Throughout the experiment there were no indications of adverse reactions, reduction in weight gain or adverse immune responses, including those animals that received 100 capsules.

Example 5: Pilot experiment (3) involving implantation of pST-L6IXS in pigs

Following example 4, investigations were conducted to assess the effect of the administering optimal pST secretion rates/capsule numbers to pigs at varying times prior to slaughter (i.e. 2, 4 and 6 weeks prior to slaughter) on back fat. 8 pigs were used for each treatment as well as 8 control (i.e. no pST capsules).

The results of the Rate of Gain measurements are provided in Figure 11.

Back fat measurements were obtained following whole carcass chilling (24 hours @ 4°C) (Figure 12). P2 measurements were recorded at the 12th rib 65mm from the centre of the spinal column. Pigs exposed to capsules secreting pST for 2, 4 and 6 weeks were observed to have significantly reduced back fat. This effect in the 2 and 6 week period is approximately a 46% reduction in back fat. The animals exposed to pST IGT capsules for 4 weeks were more variable in their back fat responses, which may relate to a possible failure to recover all the capsules from a number of these animals.

Loin muscle area in pigs exposed to secreting capsules was only significantly increased (i.e. 22 %) following 6 weeks exposure to pST IGT capsules (Figure 13).

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References:

Basic *et al*, (1996) Microencapsulation and transplantation of genetically engineered cells: A new approach to somatic gene therapy. Art. Cells, Blood subs. and Immob. Biotech 24(3): 219-255.

Magari *et al*, (1997) Pharmacological control of humanised gene therapy system implanted into nude mice. J. Clin. Invest. 100: 2865-2872.

Schrader *et al*, (1996) Identification of natural monomeric response elements of the nuclear receptor R2R/ROR. They also bind to COUP-TF homodimers. J. Biol. Chem. 271:19732-19736.

Claims:

1. An expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding a polypeptide.

2. An expression cassette according to claim 1, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

3. An expression cassette according to claim 1, wherein the insulin secretory signal is a modified insulin secretory signal having substantially the same overall biological activity as an insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1.

4. An expression cassette according to any one of claims 1 to 3, wherein the heterologous sequence encodes a polypeptide selected from hormones, cytokines, receptor agonists, receptor antagonists, pheromones, and enzymes.

5. An expression cassette according to claim 4, wherein the polypeptide is a growth hormone.

6. An expression cassette according to claim 5, wherein the polypeptide is somatotropin.

7. An expression cassette according to any of claims 1 to 6, further including one or more regulatory elements to enable pulsatile expression of the heterologous sequence .

8. A vector including an expression cassette according to any one of claims 1 to 7.

9. A recombinant cell which includes an expression cassette according to any one of claims 1 to 7.

10. A recombinant cell according to claim 9, wherein the cell is a bacterial, yeast, insect or mammalian cell.

11. A recombinant cell according to claim 10, wherein the cell is a mammalian cell.

12. A mammalian cell according to claim 11, wherein the cell is a rat myoblast (L6) cell.

13. A method of producing a polypeptide which includes culturing a recombinant cell of any one of claims 9 to 12 under conditions enabling the expression and secretion of the polypeptide and optionally isolating the polypeptide.

14. A capsule for implantation in a host, the capsule including a semi-permeable membrane encapsulating recombinant cells according to any one of claims 9 to 12.

15. A capsule according to claim 14, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

16. A method of administering a polypeptide to a host, wherein said method includes administering to the host an expression cassette according to any one of claims 1 to 7.

17. A method of administering a polypeptide to a host, wherein the method includes implanting in the host a capsule according to claim 14 or 15.

18. A method according to claim 16 or 17, wherein the host is an animal or human.

19. A method according to claim 18, wherein the host is a livestock animal.

20. A method according to claim 19, wherein the livestock animal is a pig.

21. A method of administering somatotropin to a pig, wherein the method includes implanting in the pig a capsule including a semi-permeable membrane encapsulating recombinant cells, said recombinant cells including and expressing an expression cassette including a sequence encoding an insulin secretory signal operably linked to a heterologous sequence encoding somatotropin, wherein said membrane is permeable to the expresssed somatotropin.

22. A method according to claim 21, wherein the insulin secretory signal has the amino acid sequence shown as SEQ ID NO:1.

23. A method according to claim 21, wherein the insulin secretory signal is a modified insulin secretory signal having substantially the same overall biological activity as an insulin secretory signal having the amino acid sequence shown as SEQ ID NO:1.

24. A method according to any one of claims 21 to 23, wherein the recombinant cells are mammalian cells.

25. A method according to claim 24, wherein the mammalian cells are rat myoblast (L6) cells.

26. A method according to any one of claims 21 to 25, wherein the semi-permeable membrane is an alginate-poly-L-lysine-alginate (APA) membrane.

27. A method according to any one of claims 21 to 26, wherein the pig is implanted with one or more capsules sufficient to achieve secretion of somatotropin of at least 30 ng/ml.

FIGURE 1: ISS-pST gene construct

```
   1  GCTAGCATGG CCCTGTGGAT GCGCCTCCTG CCCCTGCTGG CGCTGCTGGC
  51  CCTCTGGGGA CCTGACCCAG CCGCAGCCCT CGAGATGTTT CCAGCTATGC
 101  CACTTTCTTC TCTGTTCGCT AACGCTGTTC TTCGGGCCCA GCACCTGCAC
 151  CAACTGGCTG CCGACACCTA CAAGGAGTTT GAGCGCGCCT ACATCCCGGA
 201  GGGACAGAGG TACTCCATCC AGAACGCCCA GGCTGCCTTC TGCTTCTCGG
 251  AGACCATCCC GGCCCCCACG GGCAAGGACG AGGCCCAGCA GAGATCGGAC
 301  GTGGAGCTGC TGCGCTTCTC GCTGCTGCTC ATCCAGTCGT GGCTCGGGCC
 351  CGTGCAGTTC CTCAGCAGGG TCTTCACCAA CAGCCTGGTG TTTGGCACCT
 401  CAGACCGCGT CTACGAGAAG CTGAAGGACC TGGAGGAGGG CATCCAGGCC
 451  CTGATGCGGG AGCTGGAGGA TGGCAGCCCC CGGGCAGGAC AGATCCTCAA
 501  GCAAACCTAC GACAAATTTG ACACAAACTT GCGCAGTGAT GACGCGCTGC
 551  TTAAGAACTA CGGGCTGCTC TCCTGCTTCA AGAAGGACCT GCACAAGGCT
 601  GAGACATACC TGCGGGTCAT GAAGTGTCGC CGCTTCGTGG AGAGCAGCTG
 651  TGCCTTCTAG TCTAGA   (SEQ ID NO:3)
```

ATG...GCC- insulin secretory signal.

GCTAGC- *Nhe* I restriction site incorporated into construct in order to ligate into plasmid.

CTCGAG- *Xho* I restriction site incorporated into construct in order to ligate secretory signal and pST.

TCTAGA- *Xba* I restriction site incorporated into construct in order to ligate into plasmid.

FIGURE 2: ISS-pST peptide sequence.

```
  1  MALWMRLLPL LALLALWGPD PAAALEMFPA MPLSSLFANA VLRAQHLHQL
 51  AADTYKEFER AYIPEGQRYS IQNAQAAFCF SETIPAPTGK DEAQQRSDVE
101  LLRFSLLLIQ SWLGPVQFLS RVFTNSLVFG TSDRVYEKLK DLEEGIQALM
151  RELEDGSPRA GQILKQTYDK FDTNLRSDDA LLKNYGLLSC FKKDLHKAET
201  YLRVMKCRRF VESSCAF    (SEQ ID NO:2)
```

MAL....AAA- insulin secretory signal, cleaved upon secretion of pST.
LE- function of XhoI cleavage site; result in no predicted secondary structural changes to pST.

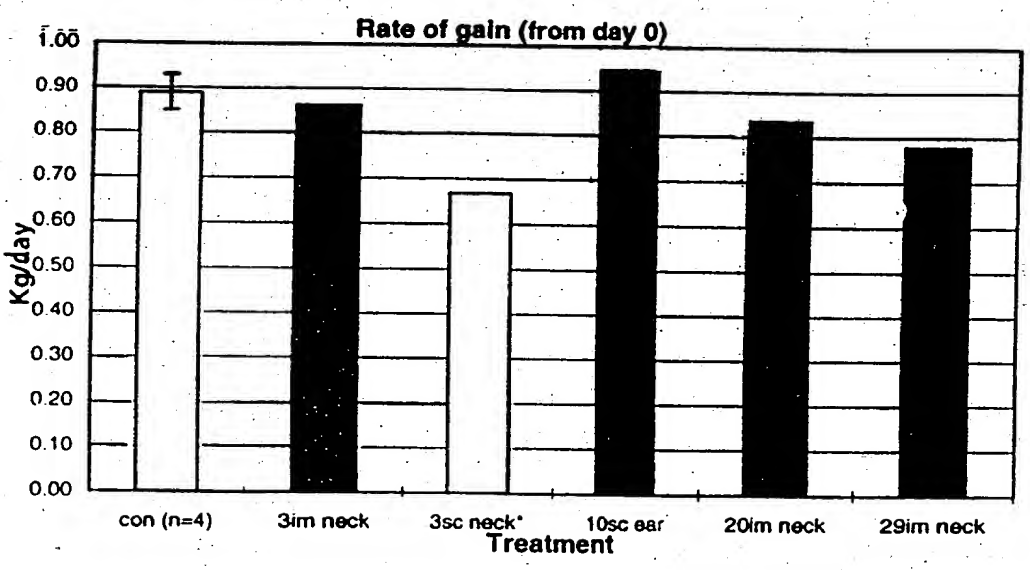

Rate of gain (from day 0)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Kg/day
con (n=4)
3im neck
3sc neck*
10sc ear
20im neck
29im neck
Treatment

Figure 3

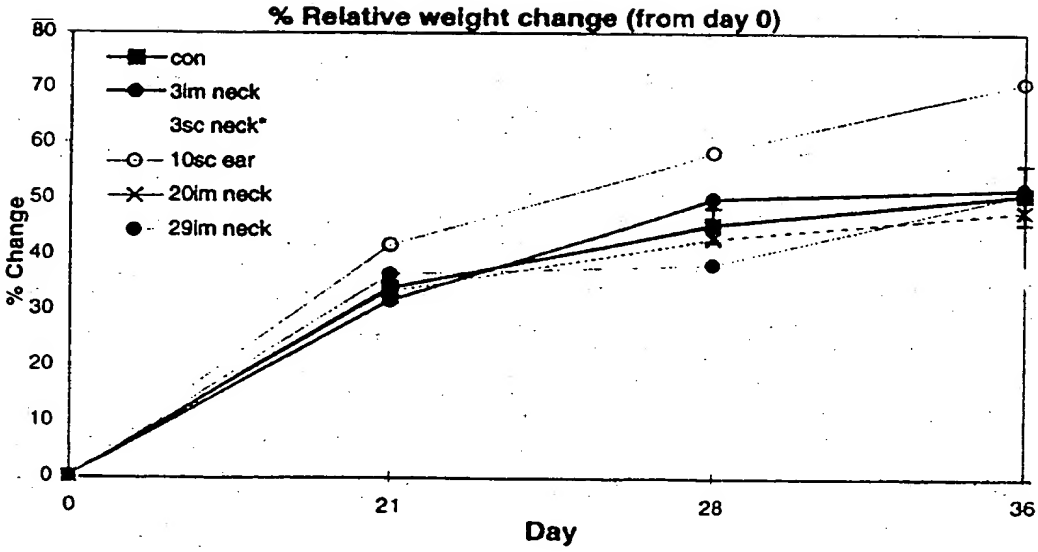

% Relative weight change (from day 0)
% Change
Day
con
3im neck
3sc neck*
10sc ear
20im neck
29im neck
0
10
20
30
40
50
60
70
80
0
21
28
36

Figure 4

Figure 5

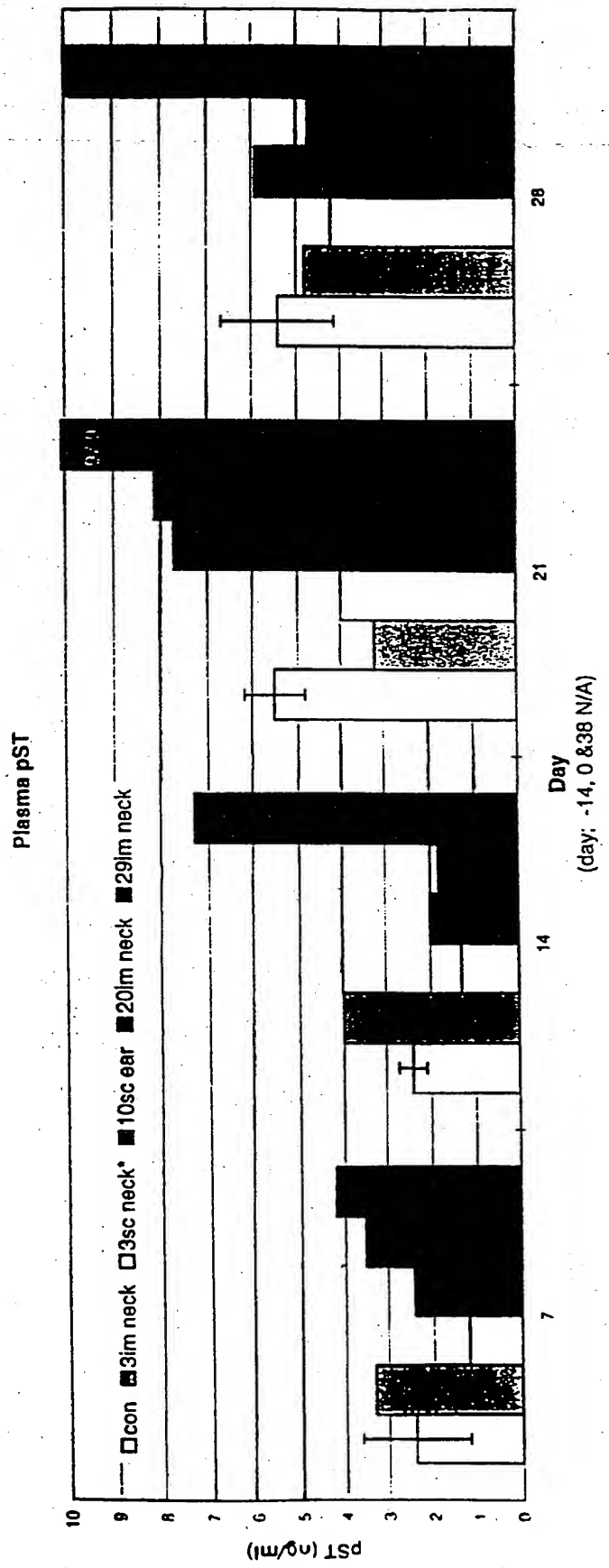

Plasma pST
Day
(day: -14, 0 &38 N/A)
pST (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
7
14
21
28
Dcon
3im neck
3sc neck*
10sc ear
20im neck
29im neck

Plate 1

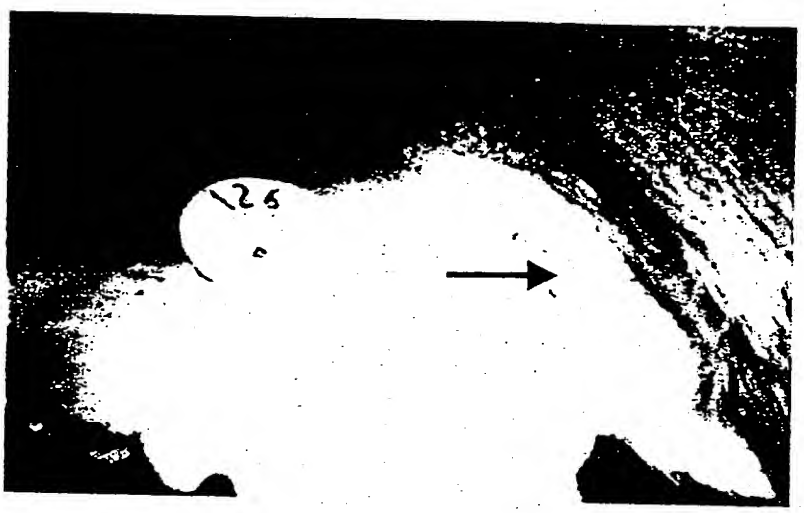


Plate 2

Figure 6

Figure 7

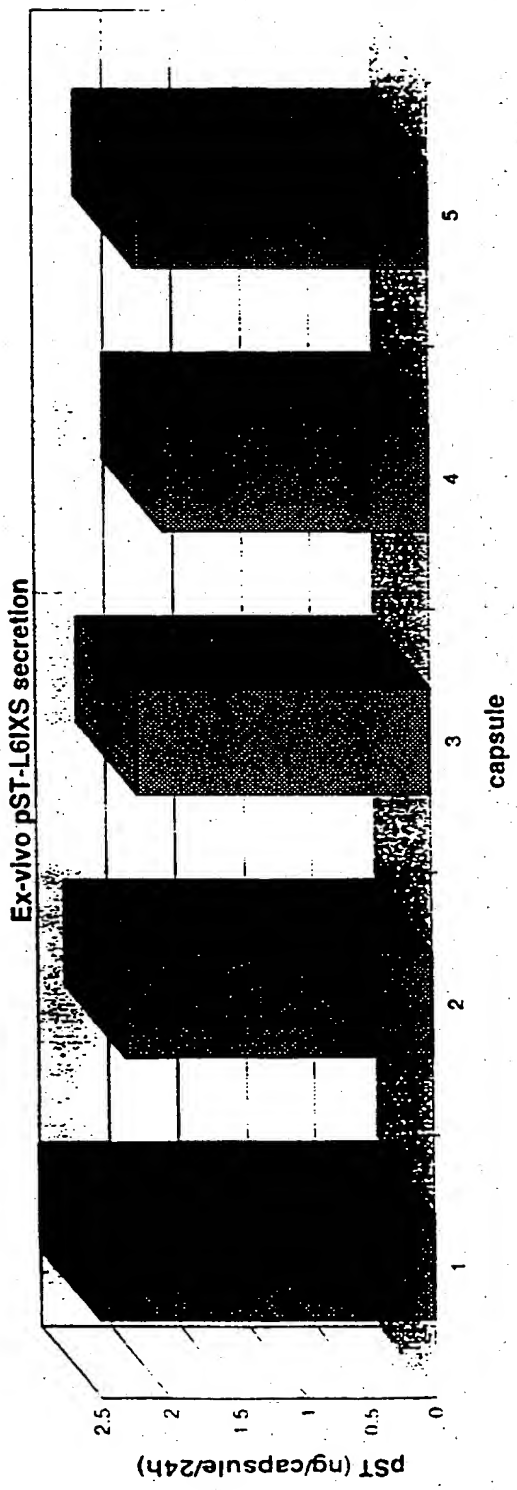

Ex-vivo pST-L6IXS secretion
pST (ng/capsule/24h)
0
0.5
1
1.5
2
2.5
capsule
1
2
3
4
5

Figure 8. Mean plasma pST (over 3 hours @ 30min intervals) before (white bars) and 1 week post pST capsule administration (black bars)(* significant).

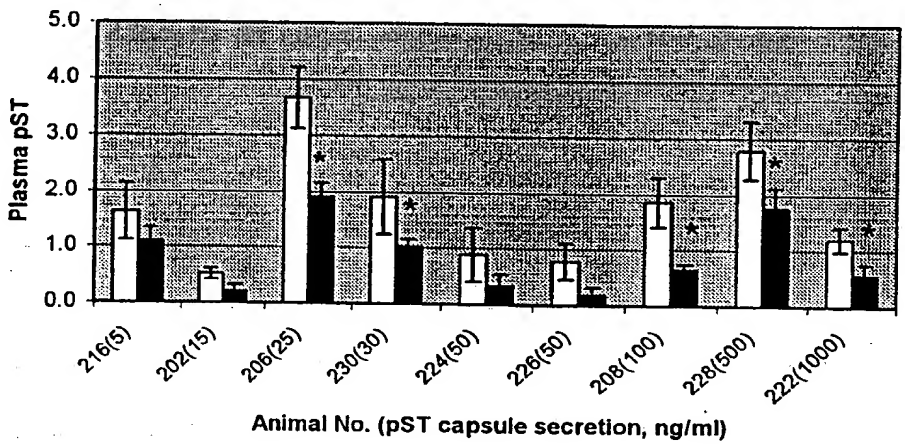


Figure 9. Daily pST concentrations

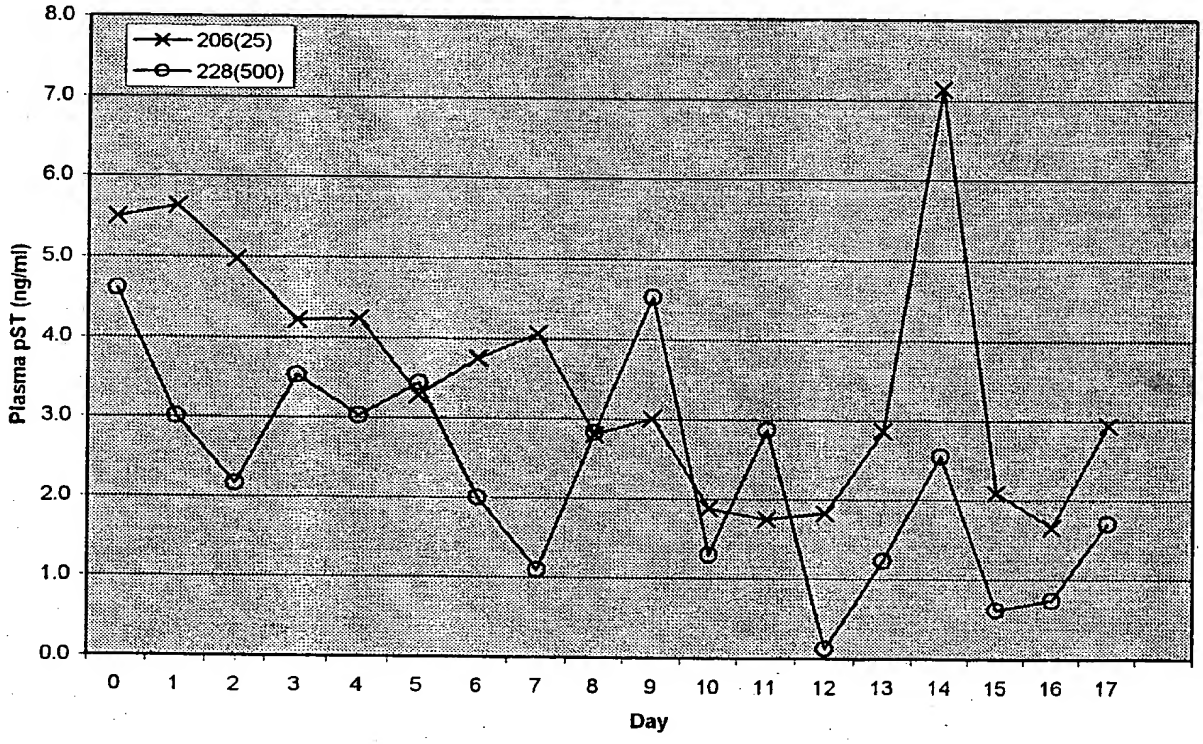


FIGURE 10

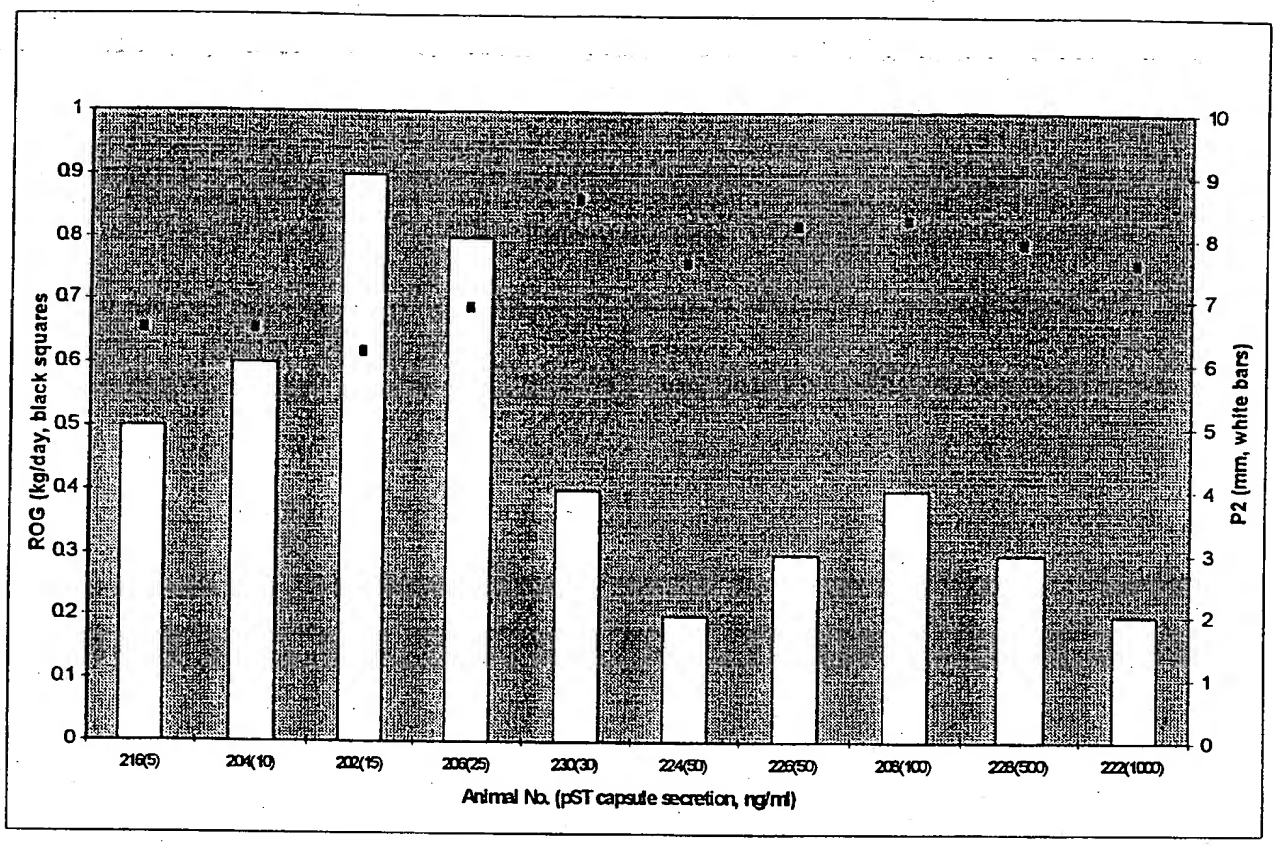

ROG (kg/day, black squares
P2 (mm, white bars)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
1
2
3
4
5
6
7
8
9
10
216(5)
204(10)
202(15)
206(25)
230(30)
224(50)
226(50)
208(100)
228(500)
222(1000)
Animal No. (pST capsule secretion, ng/ml)

FIGURE 11

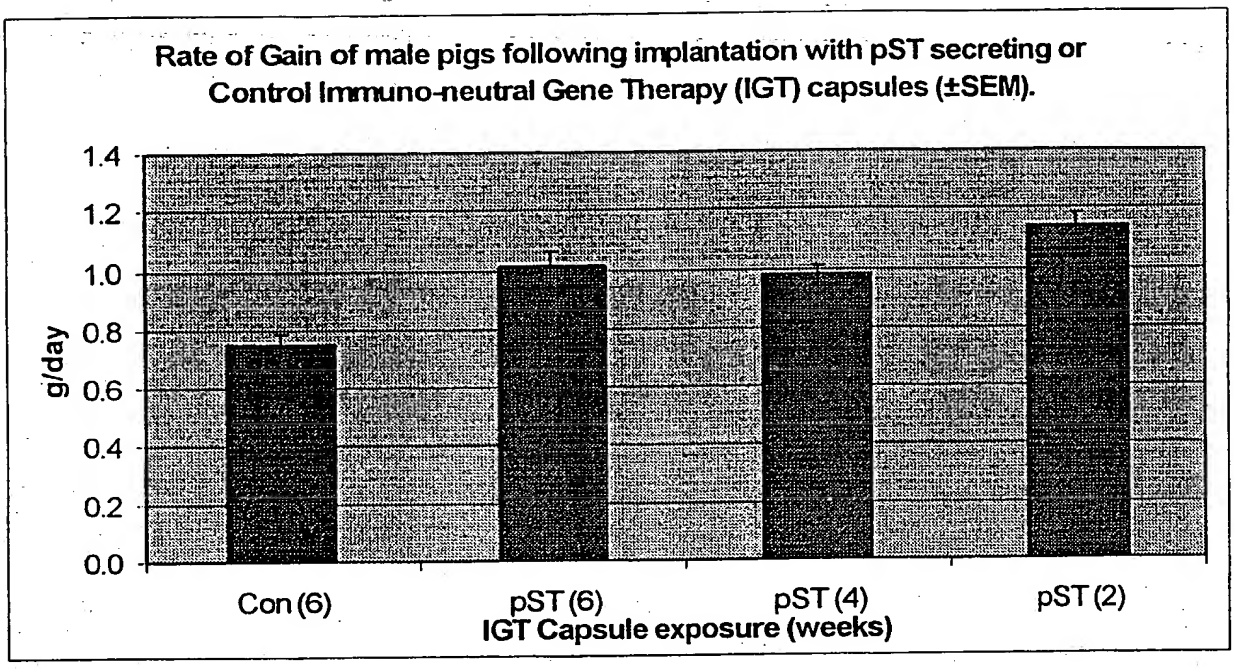

Rate of Gain of male pigs following implantation with pST secreting or Control Immuno-neutral Gene Therapy (IGT) capsules (±SEM).
g/day
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Con (6)
pST (6)
pST (4)
pST (2)
IGT Capsule exposure (weeks)

FIGURE 12

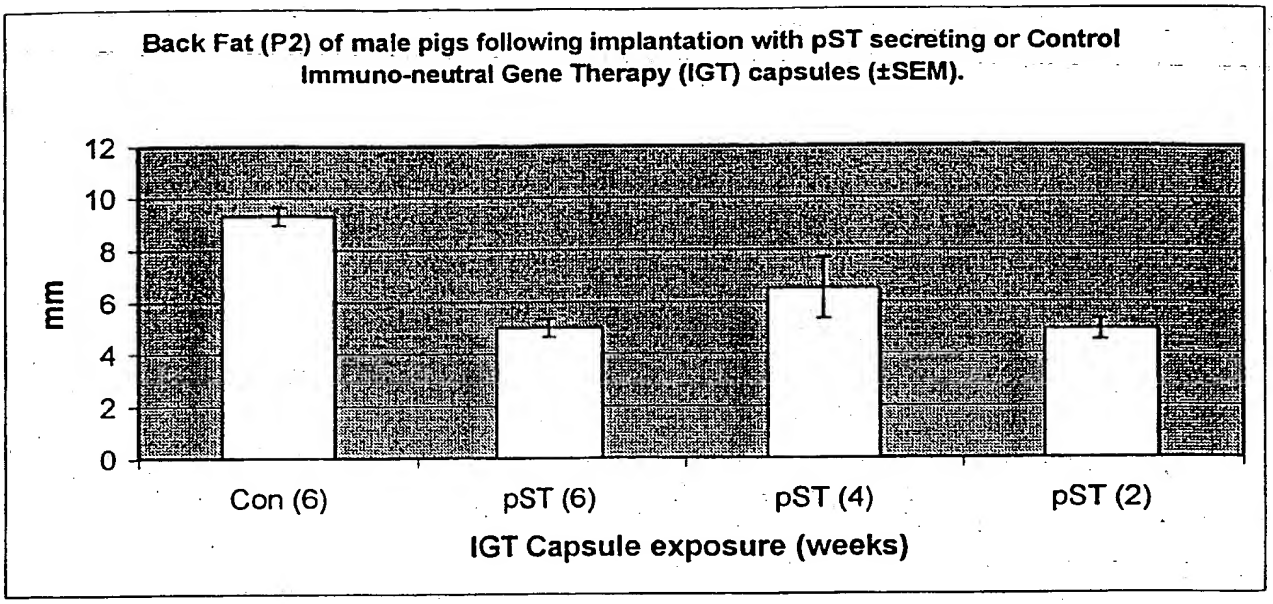
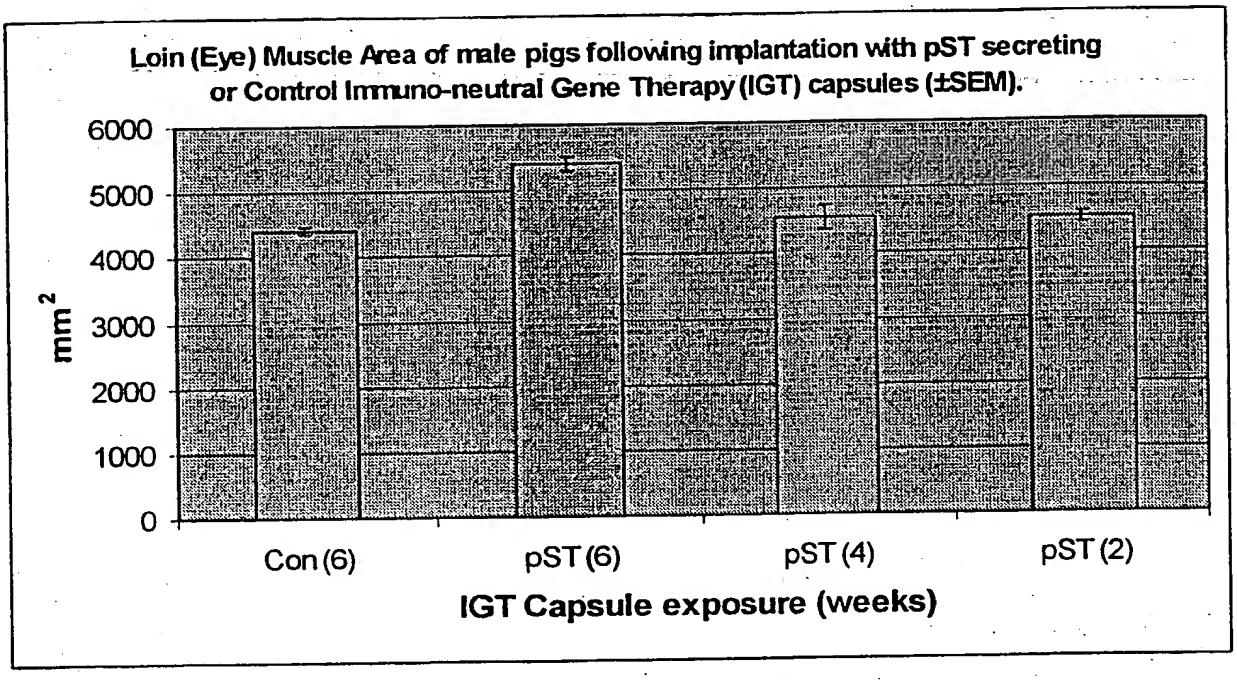

Back Fat (P2) of male pigs following implantation with pST secreting or Control Immuno-neutral Gene Therapy (IGT) capsules (±SEM).
mm
12
10
8
6
4
2
0
Con (6)
pST (6)
pST (4)
pST (2)
IGT Capsule exposure (weeks)

FIGURE 13


Loin (Eye) Muscle Area of male pigs following implantation with pST secreting or Control Immuno-neutral Gene Therapy (IGT) capsules (±SEM).
6000
5000
4000
3000
2000
1000
0
mm2
Con (6)
pST (6)
pST (4)
pST (2)
IGT Capsule exposure (weeks)

Sequence listing:

Applicants: Commonwealth Scientific and Industrial Research Organisation
University of Western Sydney (Nepean)
Pig Research and Development Corporation

Title of the Invention: Delivery system for porcine somatotropin

Prior Application Number: PP 6556
Prior Application Filing Date: 1998-10-16

Number of SEQ ID NOs: 3

Software: PatentIn Ver. 2.1

SEQ ID NO: 1
Length: 72
Type: DNA
Organism: Homo sapien

Sequence: 1
atggccctgt ggatgcgcct cctgcccctg ctggcgctgc tggccctctg gggacctgac 60
ccagccgcag cc

SEQ ID NO: 2
Length: 666
Type: DNA
Organism: Artificial Sequence

Feature:
Other Information: Description of Artificial Sequence: ISS-pST gene construct

Sequence: 2

gctagcatgg ccctgtggat gcgcctcctg cccctgctgg cgctgctggc cctctgggga 60
cctgacccag ccgcagccct cgagatgttt ccagctatgc cactttcttc tctgttcgct 120
aacgctgttc ttcgggccca gcacctgcac caactggctg ccgacaccta caaggagttt 180
gagcgcgcct acatcccgga gggacagagg tactccatcc agaacgccca ggctgccttc 240
tgcttctcgg agaccatccc ggcccccacg ggcaaggacg aggcccagca gagatcggac 300
gtggagctgc tgcgcttctc gctgctgctc atccagtcgt ggctcgggcc cgtgcagttc 360
ctcagcaggg tcttcaccaa cagcctggtg tttggcacct cagaccgcgt ctacgagaag 420
ctgaaggacc tggaggaggg catccaggcc ctgatgcggg agctggagga tggcagcccc 480
cgggcaggac agatcctcaa gcaaacctac gacaaatttg acacaaactt gcgcagtgat 540
gacgcgctgc ttaagaacta cgggctgctc tcctgcttca agaaggacct gcacaaggct 600
gagacatacc tgcgggtcat gaagtgtcgc cgcttcgtgg agagcagctg tgccttctag 660
tctaga 666

SEQ ID NO: 3

Length: 217

Type: PRT

Organism: Artificial Sequence

Feature:

Other Information: Description of Artificial Sequence: ISS-pST peptide sequence

Sequence: 3

Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu
1 5 10 15

Trp Gly Pro Asp Pro Ala Ala Ala Leu Glu Met Phe Pro Ala Met Pro
20 25 30

Leu Ser Ser Leu Phe Ala Asn Ala Val Leu Arg Ala Gln His Leu His
35 40 45

Gln Leu Ala Ala Asp Thr Tyr Lys Glu Phe Glu Arg Ala Tyr Ile Pro
50 55 60

Glu Gly Gln Arg Tyr Ser Ile Gln Asn Ala Gln Ala Ala Phe Cys Phe
65 70 75 80

Ser Glu Thr Ile Pro Ala Pro Thr Gly Lys Asp Glu Ala Gln Gln Arg
85 90 95

Ser Asp Val Glu Leu Leu Arg Phe Ser Leu Leu Leu Ile Gln Ser Trp
100 105 110

Leu Gly Pro Val Gln Phe Leu Ser Arg Val Phe Thr Asn Ser Leu Val
115 120 125

Phe Gly Thr Ser Asp Arg Val Tyr Glu Lys Leu Lys Asp Leu Glu Glu
130 135 140

Gly Ile Gln Ala Leu Met Arg Glu Leu Glu Asp Gly Ser Pro Arg Ala
145 150 155 160

Gly Gln Ile Leu Lys Gln Thr Tyr Asp Lys Phe Asp Thr Asn Leu Arg
165 170 175

Ser Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Ser Cys Phe Lys
180 185 190

Lys Asp Leu His Lys Ala Glu Thr Tyr Leu Arg Val Met Lys Cys Arg
195 200 205

Arg Phe Val Glu Ser Ser Cys Ala Phe
210 215

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00896

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/63, 15/70, 15/79, 15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
AS ABOVE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CA, MedLine — Genbank, EMBL, PDB
Insulin, signal peptide, gene expression, vector or cassette — SEQ ID 1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Cullen, Bryan R. Expression of a cloned human interleukin-2 DNA is enhanced by the substitution of a heterologous mRNA leader region. DNA. 1988. 7(9):645-650	1-20

☐ Further documents are listed in the continuation of Box C

☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 1999	-2 DEC 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer GILLIAN ALLEN Telephone No.: (02) 6283 2266